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Review

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Cold autoimmune hemolytic anemia

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ABSTRACT

Cold agglutinins are antibodies that recognize antigens on erythrocytes at temperatures below normal body temperature. Antibodies are of IgM nature and bind to "I" or "i" antigens on red blood cells, causing agglutination in red blood cells. This situation results in anemia by creating extravascular hemolysis. If there is no underlying disease, it is called primary or idiopathic cold agglutinin disease (CAD), and if there is, it is called secondary cold agglutinin syndrome (CAS). Primary CAD is an extremely rare disease, with an incidence and prevalence of 1 per million and 16 per million, respectively. It is seen twice more in women than in men, and the median age of diagnosis is 67 (between 30-92 years). The etiology of CAS includes infections, autoimmune and lymphoproliferative diseases. There are cold-related symptoms and symptoms of anemia in the clinic. In treatment, it is necessary to avoid cold in order to reduce cold-induced symptoms and hemolysis. Currently, the most effective treatment for reducing antibody production is rituximab. It can be given alone or in combination with bendamustine, interferon alfa, fludarabine and prednisolone. bortezomib is used when rituximab is ineffective or contraindicated. Plasmapheresis or intravenous immunoglobulin can be given when there is critical hemolysis or when the effectiveness of immunosuppressive therapy may start late. Treatment in CAS is the treatment of the underlying disease.

Keywords: Anemia, bortezomib, cold agglutinins, hemolytic anemia, rituximab

INTRODUCTION

CAD is an autoimmune hemolytic anemia that develops against "I" and "i" antigens on erythrocytes at temperatures below normal body temperature.[1,2] Cold agglutinins were first described by Landsteiner in 1903.[3] Clough and Richter described the relationship of cold agglutination with respiratory tract infections in 1918.[4] In 1943, Horstmann and Tatlock reported that they detected cold agglutinin in patients with primary atypical pneumonia.[5] The term CAD was first defined by Schubothe in 1966.[6]

PATHOPHYSIOLOGY

It reacts with RBC antigens with polysaccharide epitopes on glycoproteins and glycolipids, such as cold agglutinins, ceramides or glycophorins. Antigens are "I" or "i" antigens on erythrocytes. About 99% of the population is "I" positive. Less than 1% are "i" positive and these individuals are also referred to as "I" negatives. Antibodies developed against these antigens are in IgM structure at a rate of 90%, and they may rarely be IgG, IgA or λ light chains, but in heat-associated autoimmune hemolytic anemia (AHA), antibodies are mostly IgG in nature.[7,8] IgM can extend the distance between RBCs and it is a 1 million-Da molecule that can overcome the natural repulsive forces between cells, thus allowing spontaneous in vitro agglutination. At normal body temperature, circulating IgM does not remain bound on the RBC surface. However, when blood slides into the peripheral circulation, it cools and IgM temporarily binds to the RBC surface. Once the IgM molecule binds, it activates the complement cascade that binds C3b to the cell surface. C1 activates and activates C4 and C2 with C1esterase, which is then cleaved into C3a and C3b by C3 convertase. C3b-coated RBCs are phagocytosed by macrophages in the reticuloendothelial system, mostly Kupffer cells in the liver, resulting in extravascular hemolysis.[9] Phagocytosis is the whole cell rather than part of the cell membrane, but may also involve a portion of the cell membrane in IgG-mediated autoimmune hemolytic anemia. While spherocytes are not observed in CAD, they can be observed in warm CAD. In RBCs that do not phagocytosis and remain in circulation, when the body temperature returns to normal, IgM decomposes, but C3b remains bound and undergoes cleavage of the C3b surface to C3d, which can be detected by direct antiglobulin (Coombs) testing, and a positive Coombs test for complement occurs from the first findings pointing to CAD.[10]

TERMINOLOGY, EPIDEMIOLOGY

Cold-type autoimmune hemolytic anemia is called primary or idiopathic Cold Agglutinin Disease (CAD) if there is no underlying disease, and secondary cold agglutinin syndrome (CAS) if present.

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1. Primary CAD is a very rare disease, its incidence and prevalence are 1 per million and 16 per million, respectively.
2. The prevalence of CAS is not known exactly, but has been estimated from various case series. In a study conducted in Denmark, cold agglutinins were detected in vitro in 407 (82%) of 496 subjects with M. pneumoniae infection, but it was not correlated with clinical symptoms or hemolysis, and most cold agglutinins were clinically silent, cold agglutinins developed in 5% of 217 individuals, but clinical hemolysis was detected in only 1.5%.[12] CAD constitutes approximately 15% of all autoimmune hemolytic anemias. It is seen twice more in women than in men, and the median age of diagnosis is 67 (between 30-92 years).

CAUSES OF SECONDARY COLD AGGLUTININ SYNDROME

The underlying diseases in CAS are examined under three main headings.

A. Infections

It most commonly occurs during EBV[13-16] and M. pneumonia17-20 infections. However, association with Legionella, Varicella,[21,22] HIV[23], Citrobacter[24], Influenza[25,26] and Rubella[27] has also been observed in case reports. Clinically significant hemolysis does not occur in the majority of patients in CAS that develop after infection. Cold agglutinins usually appear about two weeks after the onset of primary infection, subside as the infection begins to resolve, and resolve completely within two to three months.

B. Autoimmune Diseases

Systemic sclerosis (scleroderma)[28] is observed in rheumatoid arthritis[29,30] and SLE.[31,32] In Sjögren's Syndrome, while CAD is quite rare, heat-associated autoimmune hemolytic anemia is usually observed.[33-35]

C. Malignancies

In a study involving 78 patients with cold agglutinins, 65% of the patients were associated with hematological malignancies: Lymphoma was found in 40%, WM in 17%, and Chronic lymphocytic leukemia (CLL) in 8%.[36] Hematologic malignancies were observed in 78% of 89 patients with agglutinins: Monoclonal gammopathy of Uncertain Significance (MGUS) in 47%, Non-Hodgkin B-cell lymphoma in 9%, indeterminate lymphoproliferative disease in 9%, and CLL in 4%.37 Apart from hematological malignancies, sarcoma[38] has also been described in patients with carcinoma[39] and melanoma[40], although it is thought that these cases may be coincidental rather than causal relationships. When cold agglutinin is a result of malignant proliferation, its plasma concentration can be used as a tumor marker. The antibody may disappear with appropriate treatment and may reappear with recurrence of the tumor.

CLINIC

In the clinic, patients are often asymptomatic, but in the study of Berentsen et al.[2], more than 90% of patients reported cold-related symptoms.

Cold-induced Symptoms

It causes necrosis with acrocyanosis, livedo reticularis, Raynaud's phenomenon and, in severe cases, cutaneous ulceration. In addition, weakness, fatigue, exertion and resting dyspnea may occur due to anemia.

In the laboratory, anemia can be observed in the hemogram. However, if the hemolysis is mild or compensated by reticulocytosis, anemia may not occur. In large case series, mean hemoglobin levels are approximately 9 to 10 g/dL. In some cases, it has been observed to be within the normal range but as low as 4.5 g/dL.[41-43] MCV is normal, high or low, depending on the degree of reticulocytosis. However, if the sample is cooled during processing, it will falsely rise due to RBC agglutination. Leukocytes and platelets are typically normal.

Signs of Hemolysis

Reticulocyte, LDH and indirect bilirubin increase, haptoglobulin decreases. However, hemoglobinuria, which is a sign of intravascular hemolysis, is not observed. The complement-specific C3d is positive in the Coombs test, but if the cold agglutinin is accompanied by an IgG antibody, the Coombs test may also be weakly positive for IgG. This has been reported in approximately 25% of patients. In the study of Chandesris et al.[44], it was reported that anti-C3d antibodies (74%), anti-C3d antibodies + IgG (22%) and only IgG (3%) were positive. On the peripheral smear, agglutination of erythrocytes is evident, sometimes these agglutinations can also be seen in the blood tube. Typical cold agglutinin titers in CAD are quite high. The accepted threshold is > 1:64. In addition to the titer of cold agglutinin, its thermal amplitude is also important. It reflects the temperature range at which the antibody will bind to the RBC antigen. Most clinically important cold agglutinins have a thermal amplitude exceeding 28 to 30°C.[45] Thermal amplitude is more important than the titer for clinical manifestations, but is extremely difficult to measure in the laboratory.

DIAGNOSIS

Evidence of hemolysis (high reticulocyte, LDH, indirect bilirubin and low haptoglobin), positive direct Coombs test for C3d, and a cold agglutinin titer of ≥64 at 4°C are crutial. After diagnosis, infectious, autoimmune diseases and malignancies should be investigated.

Differential Diagnosis

It is necessary to keep in mind the causes of cold-related symptoms and other hemolytic anemia. Cold-related symptoms are also observed in Reynaud's phenomenon and cryoglobulinemia, but the peripheral smear does not show agglutination in RBCs and there are no signs of autoimmune hemolytic anemia. WAHA is the most frequently confused in the differential diagnosis, the most common immune hemolytic anemia, but cold-induced symptoms are absent. Also, the Coombs test is positive for IgG instead of complement, and the smear shows spherocytes but no agglutination. Paroxysmal cold hemoglobinuria is a cold-induced autoimmune hemolytic anemia associated with IgG antibody against anti-P in RBCs; The Donath-Landsteiner test is positive and there is usually a recent viral infection in the history. The Coombs test may be positive for bound complement, but the cold agglutinin titer is minimally

elevated (<1:160). Acute or delayed hemolytic transfusion reactions may also be confused, but the peripheral smear shows no agglutination of RBCs and no cold-induced symptoms.

TREATMENT

Avoiding cold, improving anemia, reducing antibody production and treating the underlying disease in CAS are the main principles. Avoidance of cold is very important to reduce cold-induced symptoms and hemolysis. It is necessary to stay away from cold environments, food and drinks. Intravenous solutions and blood products should be brought to the appropriate temperature and used before infusion. However, in order to avoid thermal hemolysis, it should not exceed 40°C. When hospitalized patients have fever, they should be treated with antipyretics and, if necessary, antibiotics instead of cooling blankets (cold application).

Patients with mild, compensated hemolysis and stable anemia do not require transfusion for the treatment of anemia, while those with severe or symptomatic anemia may require transfusion. Blood products should be brought to the appropriate temperature, and the patient, especially the extremity to which the transfusion is applied, should be kept warm. Plasmapheresis or intravenous immunoglobulin (IVIG) treatment can be applied when there is critical hemolysis or the effectiveness of immunosuppressive therapy may start late. Since cold agglutinins are intravascular antibodies in the IgM structure, plasmapheresis is quite effective. Large-scale studies are not available to evaluate the efficacy of plasmapheresis in CAD, but case reports have shown it to reduce hemolysis and improve severe symptoms.46 The American Apheresis Society has defined therapeutic apheresis as category 2 for severe CAD. While plasmapheresis can remove existing IgM, it cannot affect IgM production, so it should only be viewed as a temporary measure until other treatments are effective. In preoperative patients, it should not be done more than 1-2 days before the procedure, this time is sufficient for the antibodies not to accumulate again, and the half-life for IgM to re-accumulate is about five days. Cryofiltration apheresis is a form of plasmapheresis that allows cold agglutinins to be removed without affecting other plasma proteins, and therefore does not require a replacement fluid such as donor plasma or albumin.47 IVIG clears cold agglutinin antibodies, but there is less experience in its use as its efficacy has not been clearly demonstrated, but some case reports has been reported.[7,48]

Treatment to reduce antibody production consists of regimens containing rituximab or bortezomib that target lymphoproliferative disorder in the bone marrow. Cold agglutinins are monoclonal in CAD while polyclonal in CAS. Treatment in CAS is the treatment of the underlying disease. Especially in CAS secondary to infection, cold agglutinins resolve spontaneously 2-4 weeks after infection treatment. Unlike warm AHA, glucocorticoids and splenectomy are not effective treatments for CAD. Glucocorticoids can reduce phagocytosis, but they cannot inhibit antibody production. However, sometimes IgG may accompany, in these cases, glucocorticoids may be added to the treatment. Splenectomy is also ineffective because in CAD the main site of RBC phagocytosis is the liver, not the spleen.

Rituximab is currently the most effective treatment, although slightly less effective than warm autoimmune hemolytic anemia (WAHA) in reducing antibody production.[49] Randomized studies have not been conducted to evaluate its efficacy, but case series have reported response rates of more than 50% and most responses are partial. In a 2006 study, rituximab was used in 52 of 86 patients with CAD; response rates were observed as 67%, complete response to combined therapy was better than single agent rituximab, and rituximab-based therapy was observed to be more effective than other immunosuppressive or cytotoxic agents.[2] In another study conducted in 2013, 89 patients with CAD had response rates of 83% for rituximab-based therapy (single agent) and 79% for combination therapy. These rates were higher than most other immunosuppressive or cytotoxic agents.[37] Similar response rates have been reported with rituximab alone or in combination therapy in small series.[49-51] The recommended dose for single-agent Rituximab is 375 mg/m^2 one day a week for four weeks.

In combination therapy with rituximab, bendamustine, fludarabine, corticosteroids and interferon alfa (INF-∝) are tried. In a prospective study of 45 patients treated with the Rituximab and Bendamustine regimen, 32 patients (71%) had a response and 18 (40%) had a complete response. Grade 3 neutropenia was observed in 15 patients (33%) and infections in 5 (11%).[51] And in a prospective study of 29 fludarabine-treated patients (10 with no prior single-agent rituximab response), the response was 76%, and the complete response was 21%. However, neutropenia was observed in 14%.[52,53] In the combination of rituximab and INF-∝, INF-∝, 5 million U were administered 3 days a week. In a series of 27 patients, the response rate was 54% and no serious side effects were observed.[54]

Bortezomib is a proteasome inhibitor used in B-cell lymphoid malignancies and its use is recommended in cases where rituximab is ineffective or contraindicated. In a study of 21 patients with CAD in whom at least one treatment was ineffective for the efficacy of bortezomib[55], six (32%) of 19 evaluable patients had a response (defined as improved transfusion dependence or a 2 g/dL increase in hemoglobin level), 3 of 6 patients had a complete response. While there was a response, partial response was observed in the other 3. Two different case reports were also observed in agreement with this study.[56,57]

Apart from rituximab and bortezomib regimens, anti-complementary therapy in CAD is being investigated. In CAD, the classical complement pathway is activated and causes extravascular hemolysis. Therefore, C1q is the target in anti-complementary therapy because it has enzymatic activity and is upstream of the classical pathway component. TNT009 is a humanized monoclonal antibody targeting C1q (based on a previous mouse monoclonal antibody TNT003) evaluated for reduction of C3b-mediated extravascular hemolysis.[58-60] A peptide inhibitor of C1q, reduction of hemolysis in CAD.[61] Anti-complementary therapies, such as eculizumab targeting distal complement component C5, are not expected to reduce extravascular hemolysis associated with early complements of the classical complement pathway, although some reports have also been shown to reduce hemolysis in selected patients.[62] However, these complement-directed therapies is not expected to improve cold-related symptoms associated with RBC agglutination because agglutination is mediated by IgM molecules.

Sutimlimab is a monoclonal antibody that targets C1 and was approved for the treatment of CAD in late 2022.[63] Pegcetacoplan is a pegylated cyclic peptide C3 inhibitor.[64] In both of these new treatments, vaccination is recommended for encapsulated bacteria before starting the treatment.

CONCLUSION

Summary

- CAD is a rare autoimmune hemolytic anemia.
- When there is an underlying cause, it is called CAS.
- When cold agglutinins are detected, especially infections, autoimmune and lymphoproliferative diseases should be investigated.
- Rituximab is still the most effective agent in treatment.
- In cases where rituximab is ineffective, combination treatments and Bortezomib are tried.
- CAD should be kept in mind in patients with hemolytic anemia, cold-related symptoms, and agglutinations of erythrocytes in peripheral smear.

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